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TITLE: The Role of Beta-TrCP Ubiquitin Ligase Receptor in the Development of Breast Cancer

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14. ABSTRACT Beta-TrCP ubiquitin ligase receptor is required for activation of anti-apoptotic transcription factor NF-kappaB. Beta-TrCP activities are essential for v-Ras-mediated transformation of cells. As beta-TrCP proteins are pivotal to activation of the NF-kappaB pathway, up-regulation of NF-kappaB transactivation via an increase in beta-TrCP levels and activities may contribute to malignant transformation of cells. Under these conditions, an elevated expression of beta-TrCP is expected to promote cell transformation. Anti-apoptotic effect of NF-kappaB is suggested among the mechanisms implicated in NF-kB-driven transformation. NF-kappaB has been shown capable of blocking apoptosis induced by TNFalpha, ionizing radiation, or the chemotherapeutic agents. Inhibition of NF-kappaB activities dramatically potentiates apoptosis of cancer cells induced by various pro-apoptotic stimuli. These and other data indicate that NF-kappaB inhibiting agents could become useful adjuvants in anti-tumor therapies. We hypothesize that beta-TrCP activities are essential for development of breast cancer. To this end we will employ new transgenic mice with inducible dominant negative beta-TrCP2 (dn-bTrCP2) in mammary tissues in breast carcinogenesis model and determine whether inhibition of beta-TrCP function will abrogate development of breast tumors. Since beta-TrCP mediates ubiquitination and degradation of IkappaB in response to IKK-inducing stimuli, identifying the mechanisms of beta-TrCP function in mouse mammary tumors may potentially lead to design of the agents capable of inhibiting beta-TrCP function and effective for cancer prevention and therapy. The result of this study may significantly contribute to our understanding of the development of human breast tumors.					
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## INTRODUCTION:

Beta-transducin repeats-containing proteins ( $\beta$ -TrCP) serve as the substrate recognition subunits for the SCF <sup>$\beta$ -TrCP</sup> E3 ubiquitin ligases. These ligases ubiquitinate specifically phosphorylated substrates and play a pivotal role in the regulation of cell division and various signal transduction pathways, which, in turn, are essential for many aspects of tumorigenesis.  $\beta$ -TrCP is known to mediate degradation of proteins, which exhibit anti-growth or pro-apoptotic properties (such as Emi1, Cdc25a, I $\kappa$ B, IFNAR1), whereas  $\beta$ -TrCP-mediated degradation of proteins which promote cell growth and survival (e.g.,  $\beta$ -catenin, PRLR) is often impaired in tumors via inhibition of specific phosphorylation (reviewed in (1)).  $\beta$ -TrCP is required for activation of anti-apoptotic transcription factor NF- $\kappa$ B by activated v-Ras and v-RAF.  $\beta$ -TrCP activities are essential for v-Ras-mediated transformation of NIH/3T3 cells (2). As  $\beta$ -TrCP proteins are pivotal to activation of the NF- $\kappa$ B pathway, up-regulation of NF- $\kappa$ B transactivation via an increase in  $\beta$ -TrCP levels and SCF <sup>$\beta$ -TrCP</sup> activities may contribute to malignant transformation of mammary epithelial cells (in addition to activation of IKK). Under these conditions, an elevated expression of  $\beta$ -TrCP is expected to promote cell transformation via activation of NF- $\kappa$ B-dependent survival pathways. Over-expression of  $\beta$ -TrCP2 was observed in most of the cell lines derived from human breast tumors as compared to the non-transformed cell lines, and tumor tissue samples from the patients with primary breast cancers (2).  $\beta$ -TrCP2 expression and activities are induced by oncogenes which activate MAPK pathway (including v-Ras and v-RAF), and this pathway is often activated in breast cancers (2). Recent data also demonstrate that inhibition of  $\beta$ -TrCP activities promotes the sensitivity of breast cancer cells to anti-growth and pro-apoptotic effects of anti-cancer drugs (3). In addition, mammary glands of  $\beta$ Trcp1(-/-) female mice display a hypoplastic phenotype; conversely, the mammary epithelia of MMTV- $\beta$ Trcp1 mice proliferate more and show increased NF- $\kappa$ B DNA binding activity and higher levels of nuclear NF- $\kappa$ B p65/RelA (4).  $\beta$ -TrCP plays an important role in protecting human breast cancer cells from apoptosis. Thus, we are interested to determine whether  $\beta$ -TrCP function is essential in mouse mammary carcinogenesis. We **hypothesize** that  $\beta$ -TrCP activities are essential for development of breast cancer. To this end we will employ new transgenic mice with inducible dominant negative  $\beta$ -TrCP2 (dn $\beta$ -TrCP2) in mammary tissues in a standard DMBA breast carcinogenesis model and determine whether inhibition of  $\beta$ -TrCP function will abrogate development of breast tumors.

## BODY:

We have encountered unanticipated problems with the breeding of our transgenic mice. Although we had re-established our breeding colony, and are now working to generate K5.rTA; TRE-dn-beta-TrCP2 double transgenic animals (Task 1), the tumor experiments proposed in the Task 2 had to be delayed. The no-cost extension approved by US Army Medical Research and Material Command will allow us to complete the Task 2 in which we will establish the role of SCF <sup>$\beta$ -TrCP</sup> ubiquitin ligase in mammary tumorigenesis.

**KEY RESEARCH ACCOMPLISHMENTS:** N/A

**REPORTABLE OUTCOMES:** N/A

**CONCLUSION:** N/A

**REFERENCES:**

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**APPENDICES:** N/A

**SUPPORTING DATA:** N/A